respectively) and reduced by F4 (to 2.7±0.4 and 0.8±0.4 the amount that of non-treated cells, respectively; FIG. **23**).

DISCUSSION

[0305] Here the inventors present in vitro indications for anti-inflammatory activity of the ethanol extract of *E. crassifolium* tubers on HaCaT normal keratinocyte cell line. The inventors also demonstrated that the different compounds detected in the EE interact to convey the reduction in IL-8 levels.

[0306] The inventors found the EE to reduce IL-8 levels induced by TNF-α. The inventors next sought to examine the effect of the EE and active fractions (F3 and F4) on inflammation that was induced in these skin cells by UVB light, an abundant natural inducer. Both acute and long-term exposure to UVB radiation causes inflammation and may induce skin cancer. UVB exposure also leads to TNF-α and IL-8 upregulation in the epidermis of normal human skin. The inventors found that the EE and to a lesser extent F4, but not F3, had a significant impact against UVB-induced inflammation in skin cells. The fact that the EE is more active than its separated fractions under similar conditions may suggest that additional compounds or relative concentrations present in the EE, and not in F4, enhanced IL-8 levels reduction. F4 also reduced MMP3 and MMP9 gene expression. Since MMP3 and MMP9 expression is a biomarker for skin inflammation, these results further validate the suggested anti-inflammatory activity of the *E. crassifolium* tubers extract.

[0307] F4 isolated from the EE was found to contain relative high concentrations of EGC, a polyphenol. Plant-derived polyphenols contain aromatic ring(s) bearing one or more hydroxyl group(s) are found in many consumed food plants such as tea, cocoa, grape, apple, blueberry, peach and orange. Polyphenols have been proposed to have many health benefits, including lessening diabetes, providing anti-cancer, anti-allergenic, anti-artherogenic, anti-inflammatory, and anti-microbial activities, as well as playing a cardio-protective role. Multiple previous studies have shown, in both in vitro and in vivo models, that tea polyphenols are effective scavengers of reactive oxygen species and may function as anti-oxidants [20]. In this study the inventors showed by an in vitro assay that the EE and fractions (F3 and F4) have anti-oxidative effects, although lower than those of green tea.

[0308] More specifically, EGC is part of a large group of catechins that have many health benefits. For example, green tea (*Camellia sinensis*) extracts, which have a long history of safe and beneficial human consumption contain the polyphenols (−)-epigallocatechin gallate (EGCG) as the most abundant compound, followed by (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin and (−)-catechin. All these compounds from green tea, except for catechin, reduced IL-8 production in human nasal fibroblasts and A549 epithelial cells. The inventors therefore sought to determine whether EGC is one of the anti-inflammatory active molecules in the EE.

[0309] The inventors found that the commercial standard EGC had lower ability to reduce IL-8 levels in cells in comparison to F4. These results suggest that additional compounds in F4 may confer EGC with better activity. Other compounds found in F4 include gallic acid, trans-catechin and cis-catechin, along with palmitic acid and stearic acid. In addition, when trans- and cis-catechins were removed from the active fraction (as in F4-6), activity was reduced. It is, therefore, possible that although purified catechin is not active on its own, the presence of trans- and cis-catechins is needed to enhance EGC and gallic acid anti-inflammatory activity. Indeed, activity of a combination of EGC, gallic acid and catechin at similar relative amounts and concentrations as found in F4 was higher than that of the individual compounds. A similar trend of increased anti-oxidant activity of a combination of these compounds present in total extracts made from grape seeds and skins or green or Labrador tea was reported previously. However, the EE and F4 activity was still higher than that of the combination produced from pure compounds, suggesting that additional compounds are present in the plant and contribute to its high overall anti-inflammatory activity.

[0310] The inventors have shown that an ethanol extract of *E. crassifolium*, a plant used in folk-medicine plant, has substantial in vitro anti-inflammatory and anti-oxidative activities. EGC and additional phenolic compounds are suggested to be some of the active compounds in this extract, whereas certain combinations of these compounds present in the plant extract led to increased activity. The anti-oxidative activity of the EE and its ability to suppress UVB-induced increase in IL-8 level suggest it to be a noteworthy candidate for inclusion in products for skin treatment and protection.

[0311] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

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